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Electrospun Fibers as a Solid-State Real-Time Zinc Ion Sensor with High Sensitivity and Cell Medium Compatibility

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***meso*-2,6-Dichlorophenyltripyrrinone (TPN-Cl_2), a probe molecule for zinc II ions, is dispersed in a polymer host. The red fluorescence peak at 620 nm appears when the molecule forms a complex with zinc at its center. TPN-Cl_2 has a high selectivity for zinc II and tolerates many common metal ions present in the human body. The probe molecules are blended with a hydrogel polymer, poly(2-hydroxyethyl methacrylate) (poly HEMA), with 30 wt% dimethylformamide (DMF). The fiber structure with 1 μm diameter is made by electrospinning in DMF solution of the probe and poly HEMA mixture. The fibrous film detects zinc ions with concentrations as low as 10^{-6} M in real-time both in water and in the commonly used cell culture liquid media Dulbecco's modified Eagle medium (DMEM) and fetal bovine serum (FBS), which contain many metal ions and proteins. The time-resolution is 5 min for 10^{-6} M and 1 min for 10^{-5} M. This sensitivity and response speed satisfy the requirements for non-invasive biomedical studies.**

1. Introduction

Solid-state real-time chemical sensors are important for biomedical research and medicine. Conventionally, the target chemicals are detected by dissolving the probe molecules in liquid, then the color or fluorescence changes result from the reaction with the target. The probe molecules may also interfere with the cell or tissue that releases the chemicals. In other words such a soluble probe is invasive. On the other hand a solid-state sensor is non-invasive and it transforms the real-time chemical concentration into electronic signals without interference with the biological processes under study. Zinc II ions play several key roles in animals and their pathology. Zinc ions play key roles in normal physiology and in pathological conditions.[1–3] It is a transynaptic mediator. Most Zn^{+2} in the brain is tightly bound or sequestered in cellular compartments. High levels of zinc release in the synapse contribute to the selective nerve cell injury from stroke and from Alzheimer's disease.[4,5] Zinc is the second most abundant transition metal ion in the human body after iron. Around the neural axon the zinc ion concentration is as high as 10^{-6} M. In order to study the dynamics of biological processes such as stroke, it is important to detect the zinc ion concentration every few minutes. Such real-time detection is

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DOI: 10.1002/adfm.201201242

impossible for conventional molecular probes dissolved in solution. In practice, the solid-state zinc sensor must function not in water but instead in liquid biological medium, which usually contains many other ions and proteins. Those contents of the medium should not interfere with the detection and the sensor structure should be stable for long immersion times in the medium. To date most zinc ion detection is based on soluble fluorescent molecular probes[6,7] or dispersed nanoparticles.[8] Despite their high sensitivity they are invasive and do not give real-time results. Recently we reported a solid-state zinc sensor with a fluorescent molecule in a polymer host.[9] The sensitivity of 10^{-4} M is, however, not enough for biomedical applications and the detection was measured in water rather than the complex cell buffer medium. A highly sensitive solid-state zinc sensor that is stable in the common cell buffer medium has not been reported.

Here, we developed a solid-state zinc ion sensor with electrospinning fibers containing a highly selective fluorescent probe molecule TPN-Cl_2.[10] The TPN-Cl_2 probe has red fluorescence only when it captures a zinc ion at its center. The molecules are blended with a host polymer, poly(2-hydroxyethyl methacrylate) (poly HEMA), and do not diffuse into the liquid medium after long immersion times. The polymer host containing the probe is made in fiber form using electrospinning with a fiber diameter around 1 μm. Because of the large surface area of the fiber film the zinc ion sensitivity is enhanced from 10^{-4} M for a planar film to 10^{-6} M for a fiber film. Such a concentration occurs near the neural axon region and is a criterion for the biological applications of the developed sensor. The TPN-Cl_2 probe is able to detect zinc ions with high tolerance to the presence of a wide range of metal ions. As a result, the sensor works well in both water and the complex cell buffer medium that contains many other ions. The fiber film is also highly stable in acid. The time resolution is about 1 min for a typical zinc concentration of 10^{-5} M. This rapid time response makes the sensor a possible tool to monitor rapidly changing processes, such as stroke, which have time scales of hours.

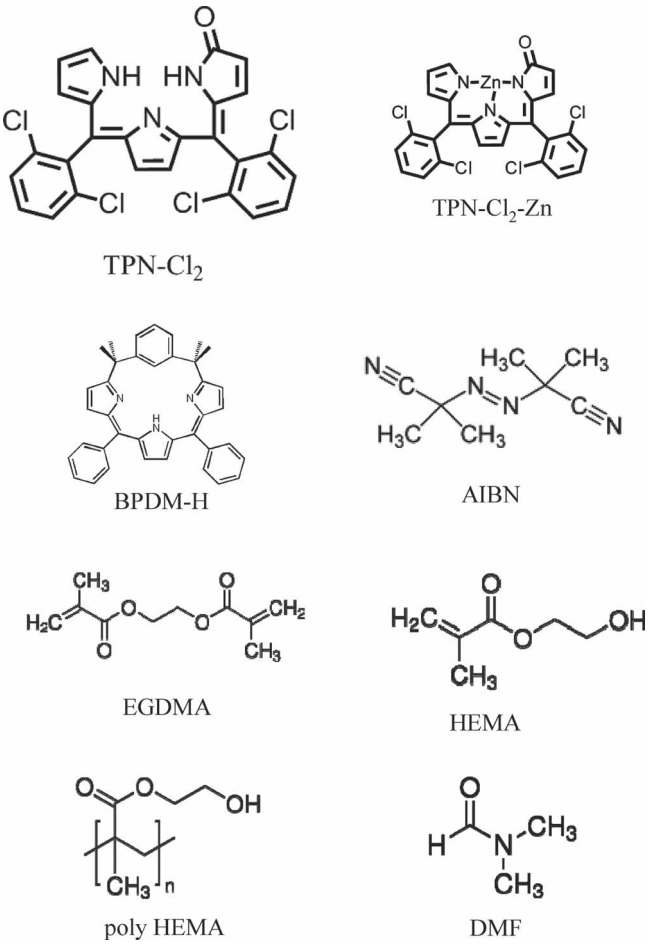

Figure 1. Chemical structures of the materials.

2. The Probe Molecule and Fiber Film

The zinc ion probe TPN-Cl_2 is shown in **Figure 1**. When a zinc ion is captured it becomes a complex, TPN-Cl_2-Zn, which is shown in Figure 1. The photoluminescence (PL) spectrum under excitation at 565 nm wavelength in methanol solution before and after Zn chelating is shown in **Figure 2**A. The red fluorescence peaked at 620 nm is present only in TPN-Cl_2-Zn. The turn-on of PL can be observed for Zn ion concentrations as low as 10^{-8} M in methanol solutions. The PL is not turned on by other common ions, as shown in Figure 2B, where the concentration of other metal ions is 2×10^{-5} M and the concentration of TPN-Cl_2 is 10^{-6} M. Furthermore, the response to Zn ions tolerates the presence of most common ions; this is shown in Figure 2B, where metal ions are added before zinc. In particular, Mg II is tolerated in contrast to the probe *m*-benziporpho dimethene (BPDM-H), which we studied previously (Figure 2C).[9] Mn II, Co II, and Ni II do interfere with zinc detection, but their concentrations in animals are very low. In an ideal solid-state sensor structure the zinc probe molecules are stably fixed in a porous host with similar fluorescence response to the zinc ions in water and in the liquid buffer medium used for cell culture. The contents of two typical liquid media, Hank's balanced salt solution (HBSS) and DMEM, are listed in **Table 1**. DMEM contains many proteins and more ions than HBSS, and it is commonly used for living cells in cultures. For example, Fe III is present in DMEM but not in HBSS. Our goal is therefore to develop a sensor that can function in DMEM, which is more challenging than HBSS.

TPN-Cl_2 molecules are blended with poly HEMA host for fiber and planar structures for comparison. For the planar structure, the monomers are mixed with the probe followed by a thermal curing to transform the mixture to a matrix of poly HEMA.[9] The resulting planar film is shown in **Figure 3**C. Electrospinning system shown in Figure 3A is used to generate the fiber structure.[11–13] Under the high voltage between the nozzle and the substrate, the solution is charged and fine fibers form due to Coulomb repulsion.[14] In order to provide enough viscosity for the electrospinning poly HEMA rather than the monomers are mixed with TPN-Cl_2 in DMF solution at 30 wt% with a molecular weight of 300 000 (300 k) for poly HEMA, and TPN-Cl_2 in DMF solution at 2.52×10^{-2} wt%. The resulting fiber film is shown in the scanning electron

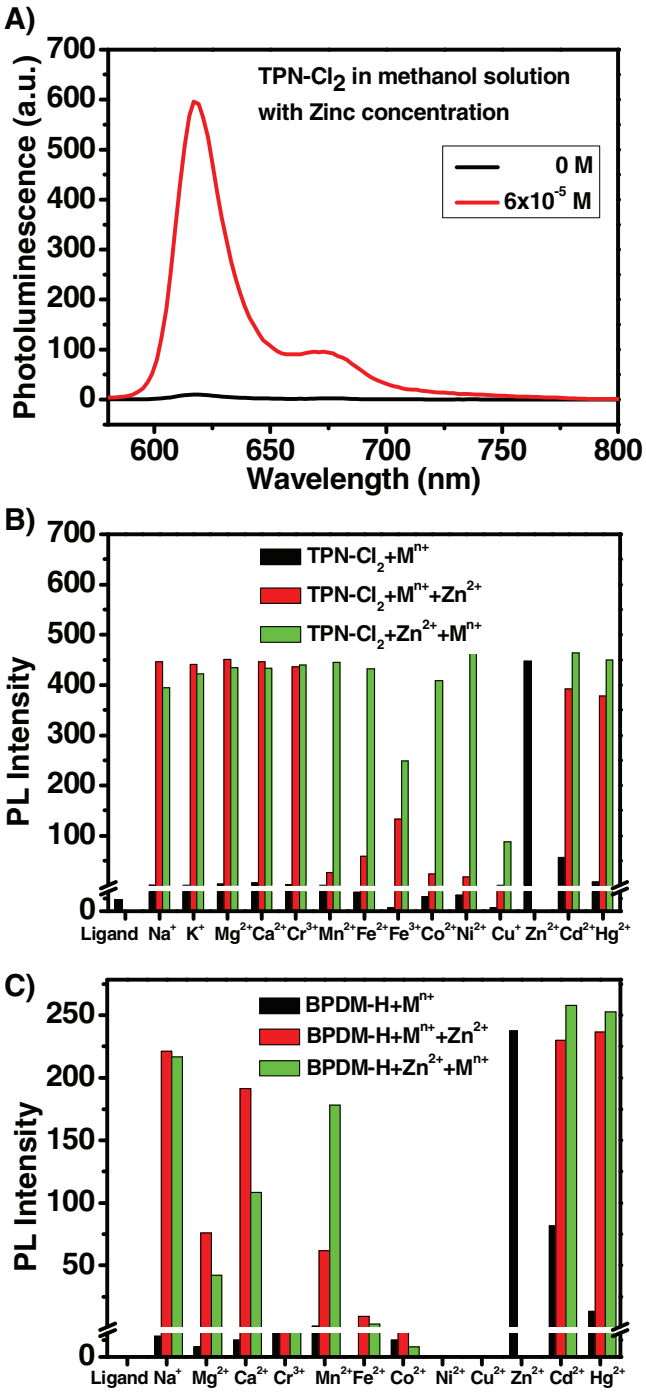

Figure 2. A) The photoluminescence (PL) spectrum of TPN-Cl_2 in methanol solution before and after Zn chelation. B) TPN-Cl_2's specificity: the concentration of TPN-Cl_2 is 10^{-6} M and the concentration of all other metal ion is 2×10^{-5} M. C) BPDM-H's specificity: the concentration of BPDM-H is 3×10^{-6} M and the concentration of all other metal ion is 6×10^{-5} M.

microscopy (SEM) images in **Figure 4**A. The fiber diameter is about 1 μm. There is some swelling of the fiber after water immersion, as shown in Figure 4B. The water permeability is expected to be reduced by the swelling. High molecular weight of poly HEMA is shown to be important to reduce the

Table 1. The content of a two typical liquid media: DMEM and HBSS.

DMEM Components	[mM]
Amino Acids	
Glycine	0.4
L-Arginine hydrochloride	0.398
L-Cystine 2HCl	0.201
L-Histidine hydrochloride-H_2O	0.2
L-Isoleucine	0.802
L-Leucine	0.802
L-Lysine hydrochloride	0.798
L-Methionine	0.201
L-Phenylalanine	0.4
L-Serine	0.4
L-Threonine	0.798
L-Tryptophan	0.0784
L-Tyrosine disodium salt dihydrate	0.398
L-Valine	0.803
Vitamins	
Choline chloride	0.0286
D-Calcium pantothenate	0.00839
Folic Acid	0.00907
Niacinamide	0.0328
Pyridoxine hydrochloride	0.0196
Riboflavin	0.00106
Thiamine hydrochloride	0.0119
i-Inositol	0.04
Inorganic Salts	
Calcium chloride ($CaCl_2$) (anhyd.)	1.8
Ferric nitrate ($Fe(NO_3)_3$-$9H_2O$)	0.000248
Magnesium sulfate ($MgSO_4$) (anhyd.)	0.814
Potassium chloride (KCl)	5.33
Sodium bicarbonate ($NaHCO_3$)	44.05
Sodium chloride (NaCl)	110.34
Sodium phosphate monobasic (NaH_2PO_4-H_2O)	0.906
Other Components	
D-Glucose (Dextrose)	5.56
Sodium pyruvate	1
HBSS Components	**[mM]**
Inorganic Salts	
Calcium chloride ($CaCl_2$) (anhyd.)	1.26
Magnesium chloride ($MgCl_2$-$6H_2O$)	0.493
Magnesium sulfate ($MgSO_4$-$7H_2O$)	0.407
Potassium chloride (KCl)	5.33
Potassium phosphate monobasic (KH_2PO_4)	0.441
Sodium bicarbonate ($NaHCO_3$)	4.17
Sodium chloride (NaCl)	137.93
Sodium phosphate dibasic (Na_2HPO_4) anhydrous	0.338
Other Components	
D-Glucose (Dextrose)	5.56

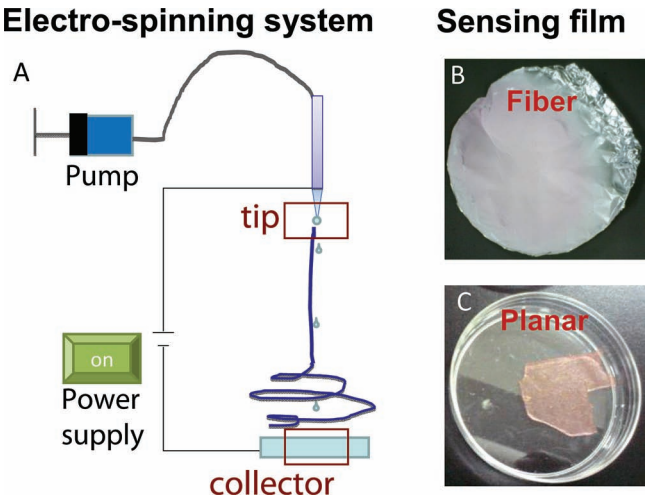

Figure 3. A) The electrospinning system. B) The fiber film on the substrate. C) The planar film.

swelling, as shown in Figure 4C,D. The fiber film on the substrate is shown in Figure 3B with a thickness around 0.87 mm. The PL spectra for the fiber and planar film in water, with or without zinc ions, are shown in **Figure 5**A–E and **Figure 6**A–C. The PL spectra for TPN-Cl_2 in solution and in the fiber are similar, with the main peak at 620 nm being responsive to the zinc ions. Before the introduction of the zinc ions, there is a clear PL background in the fiber but the background is barely detectable in solution. The fiber network may inhibit a vibration mode, which would quench the 620 nm emission of free TPN-Cl_2 in solution. The similarity of the PL spectra in solution and fiber suggests that there is no chemical reaction and the physical interactions between the fiber host and the TPN-Cl_2 probe molecules are rather weak. On the other hand the main peak of the PL spectrum of the planar film is changed to 570 nm, which does not respond to zinc ions. The 620 nm emission becomes a shoulder in the spectrum and remains responsive to zinc ions. The difference in the PL spectra in the planar structure relative to the film and to solution and fiber implies that the physical interaction is stronger and the geometry of the probe molecules is distorted. For comparison, the PL of the previous probe, BPDM-H, with zinc ions in the planar film is also shown.

3. Sensor Results and Discussions

A microfluidics system is shown in **Figure 7**, where a polydimethylsiloxane (PDMS) mold on glass is used to rapidly change the zinc ion concentration.[9] A syringe is used to inject solution with zero or various zinc concentration into the channel. The sensing film is immersed in the liquid inside the channel. The film is constantly excited by 405 nm laser

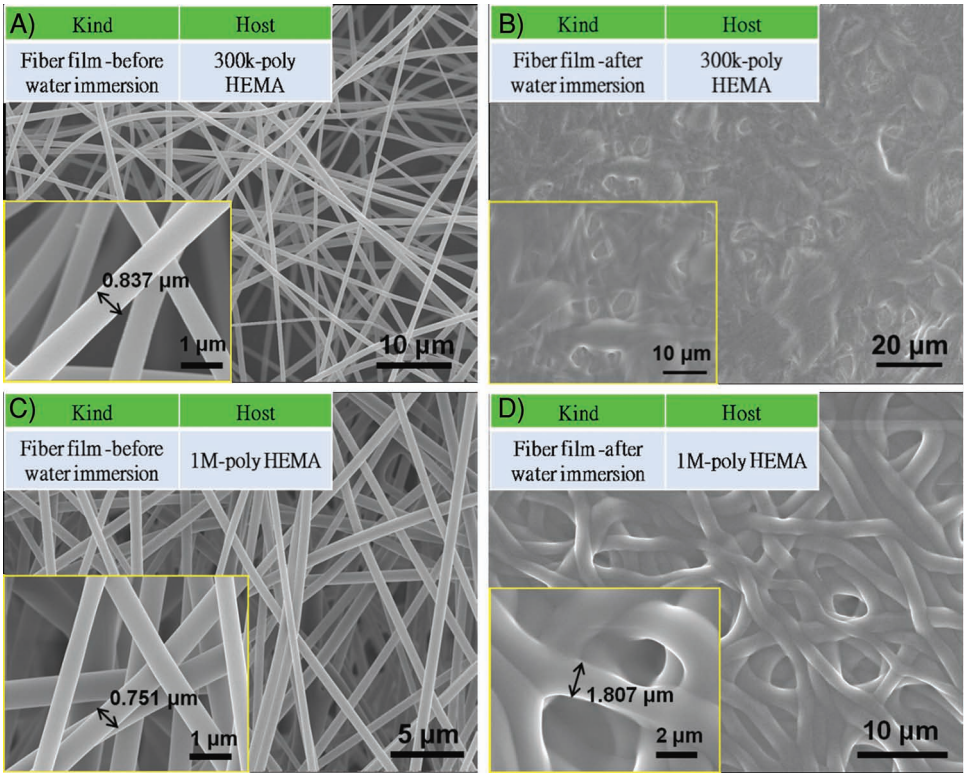

Figure 4. The SEM image of the fiber film. A) 300 k poly HEMA before water immersion. B) 300 k poly HEMA after water immersion. C) 1 M (1 million) poly HEMA before water immersion. D) 1 M poly HEMA after water immersion.

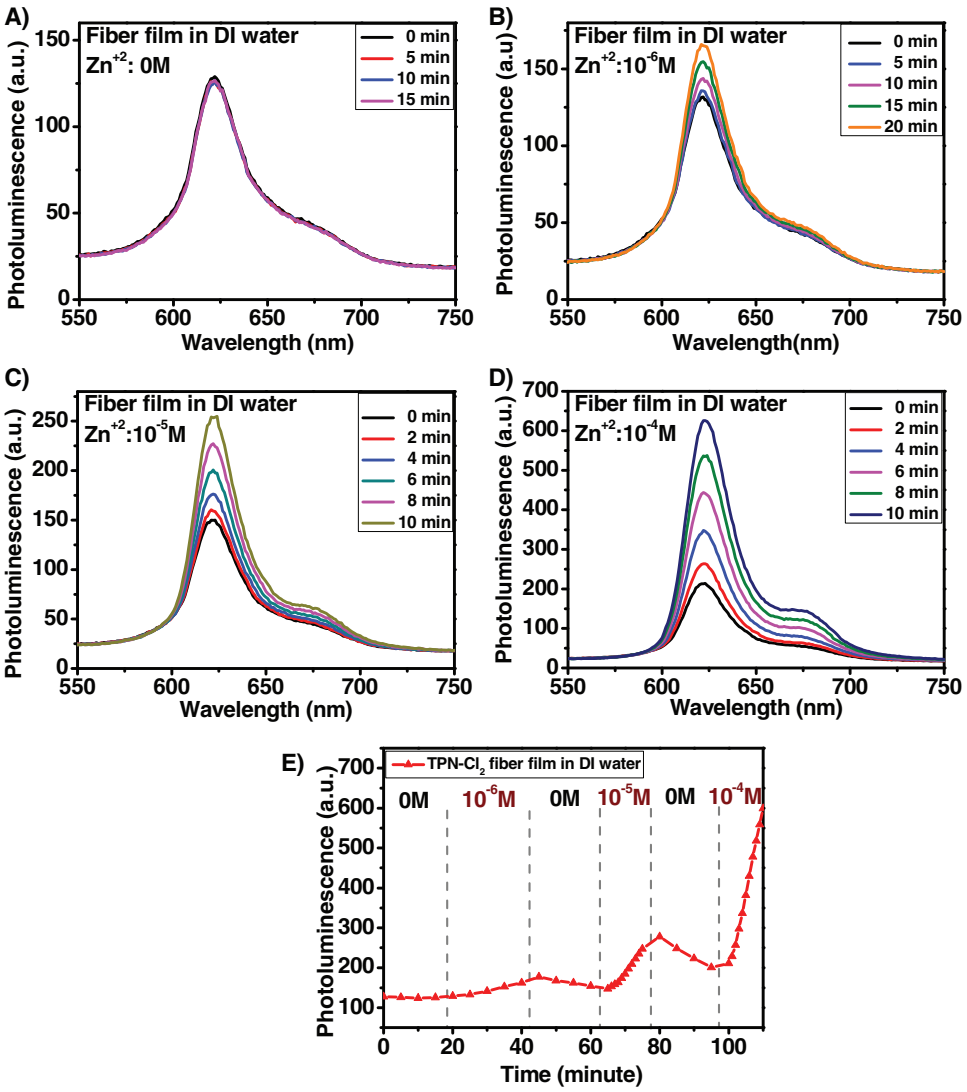

Figure 5. Time dependence of the PL spectra for the fiber film in deionized water (DI water) with zinc ions, measured with a PL spectrometer: A) 0 M zinc ions; B) 10^{-6} M zinc ions; C) 10^{-5} M zinc ions; and D) 10^{-4} M zinc ion. E) The PL spectra in DI water with different concentrations of zinc ions.

diode with pulse width of 10 s and period of 60 s. The PL is registered by a charge-coupled-device (CCD) camera. The PL signals at the peak wavelength of 620 nm are shown in **Figure 8**C–E as a function of time for the TPN-Cl_2 sensing film under different cell culture liquid media. Even though this work is focused on the probe TPN-Cl_2, the results of the previous probe BPDM-H in the same setup are presented as a comparison in order to illustrate their difference in tolerance to the culture media. The former turns out to have a far better tolerance than the latter, as shown. Consider BPDM-H first, Figure 8A is the PL spectrum of BPDM-H planar film, and Figure 8B shows that while it works well in HBSS, no response to zinc ion is observed when more than 10 vol% of DMEM is added. Presumably the Mg II in DMEM occupies the center of BPDM-H and block the zinc ions as suggested in the selectivity shown in Figure 2C above. The result for TPN-Cl_2 planar film is shown in Figure 8C. It turns out that such a film works well in pure DMEM. In standard cell culture DMEM is mixed with the fetal bovine serum (FBS) with a volume ratio 9:1. As such, FBS is added to the poly HEMA film containing TPN-Cl_2 and it still responds well to Zn II ions, as shown in Figure 8E. The functioning in DMEM plus FBS is a highly desirable property. The compatibility of TPN-Cl_2 film in DMEM demonstrates again that this probe is highly selective to zinc II and tolerates the presence of a wide range of chemicals including organic molecules and metal ions. The film has an almost exclusive response to zinc ions, even in a complex environment. The slope of the fluorescence as a function of time can be used to decide the real-time zinc ion concentration. The time resolution is defined as the interval required to

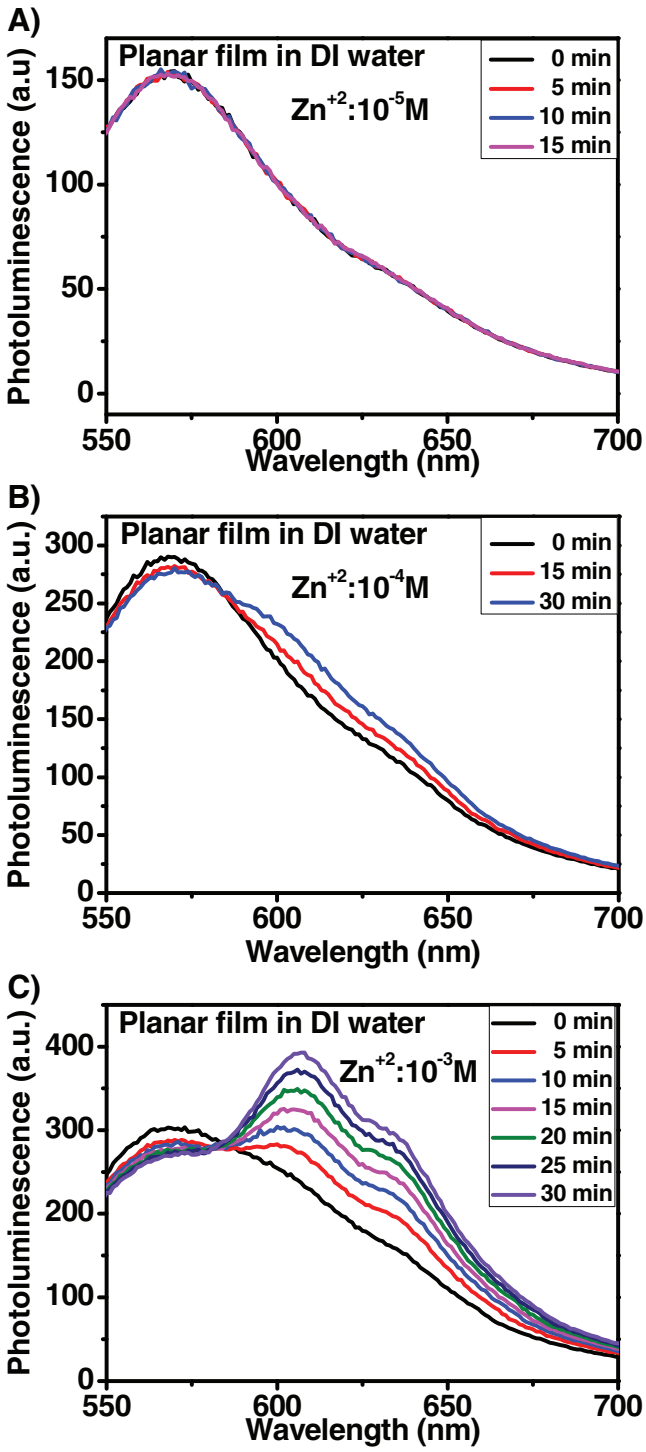

Figure 6. Time dependence of the PL spectra of the planar film in DI water with zinc ions measured with a PL spectrometer: A) 10^{-5} M zinc ions; B) 10^{-4} M zinc ions; and C) 10^{-3} M zinc ions.

identify a clear difference in the slope. For TPN-Cl_2 the time resolution is about 5 min for a zinc concentration of 10^{-4} M, both in water and pure DMEM. It can be further improved by the fiber film as described below.

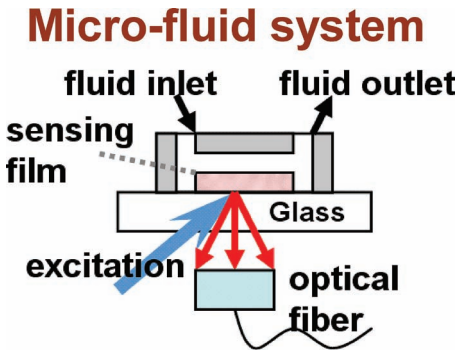

Figure 7. Microfluid system. The sensing film is the fiber or planar film with the host and probe.

So far the zinc ion detection has been done in a planar film. The detection limit is 10^{-4} M, as shown in Figure 6A–C. The detection limit of the fiber film for TPN-Cl_2 in poly HEMA host is presented in Figure 5A–E. Despite of the less swelling of the 1 million molecular weight poly HEMA shown in Figure 4B,D, no significant improvement in the sensitivity is observed as the molecular weight is increased from 300 k to 1 million. Due the larger fiber film deposition area and easier control during the electrospinning process, the 300 k poly HEMA is used in all the fiber data below unless otherwise specified. For better spectral resolution, instead of using the CCD camera, the spectra are recorded using a PL spectrometer and the film is immersed in a quartz vessel. The time dependence of Figure 5E is shown by plotting the PL value at 620 nm (recorded using a spectrometer) versus time, under changing zinc concentration in the quartz vessel. For water solution the zinc ion concentration is changed from 10^{-6} M to 10^{-4} M. The fiber film has a clear response to zinc ions for concentrations as low as 10^{-6} M. The sensitivity is therefore improved by two orders of magnitude as the film is changed from planar to fibrous with much larger surface area. Similar detection limit of 10^{-6} M is obtained from TPN-Cl_2 fiber film in pure DMEM solution as shown in **Figure 9**A–E. In Figure 9A the PL at peak wavelength of 620 nm is plotted as a function of time for various zinc ion concentrations for the fiber film. Different concentrations gives different slope values, which are used to measure the sensing. For zinc ion concentrations of 10^{-6} M the resolution is about 5 min, whereas the time resolution is raised to 1 min for a concentration of 10^{-5} M. As expected there is a trade-off between the concentration and the response time. Nevertheless 5 min resolution at concentrations as low as 10^{-6} M is expected to satisfy the requirements for some biomedical applications. The PL responses of fiber films made of different poly HEMA molecular weights are compared. Even though the level of swelling is lower in high molecular weight fiber as shown in Figure 4B,D, the sensing properties are quite similar for the two molecular weights. All the original separated fiber structures are destroyed by the immersion regardless of the molecular weight. In order to improve the film sensitivity from 10^{-6} M to 10^{-8} M free probe in solution, the fiber host with high stability in the solution may be necessary to allow for rapid zinc ion diffusion.

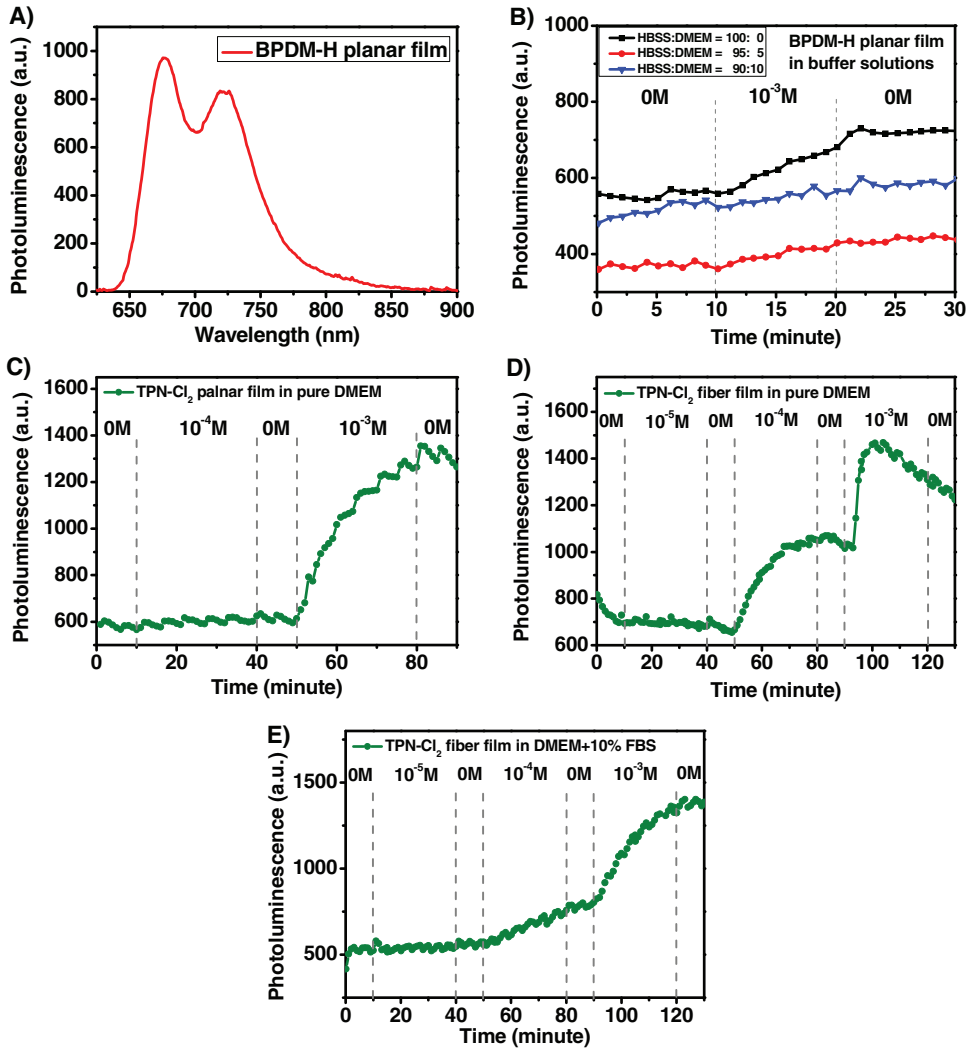

Figure 8. The PL at its peak wavelength as a function of time for the sensing film under buffer. All data were measured with a CCD camera. A) The PL spectrum of BPDM-H planar film. B) The working conditions of the BPDM-H planar film made by the buffer solutions with different ratio. The time-dependent PL is taken at 680 nm. C–E) The time-dependent PL of TPN-Cl_2 is taken at 620 nm. C) The planar film under pure DMEM. D) The fiber film under pure DMEM. E) The fiber film under DMEM + 10% FBS.

Finally we show that the TPN-Cl_2 fiber film is stable and remains responsive to zinc ion even in a very acid condition. The pH value of water is tuned by adding hydrochloric acid (HCl). The PL spectrum for various pH values is shown. For pH between 6.16 and 3.31 the PL response to zinc ion remains the similar and its intensity drops and shape changes only at pH of 2.38. The sensing film therefore works well unless the conditions are very acid (pH below 3). In the PL responses shown in Figure 5A–E above, the pH of the water solution changes slightly as zinc acetate, the source of zinc ions, is added. The pH versus time is shown in **Figure 10**F. Because the pH is always above 5 in the safe range shown in Figure 10A–D, the possibility that the PL modulation in Figure 5A–E results from pH variation is ruled out. Even though most of the human body remains neutral, some local areas may be quite acid. It is therefore useful if the sensor can operate under acid conditions. Figure 10C shows that the PL still respond well to zinc ion concentration of 10^{-3} M for the fiber film immersed in an acid aqueous solution of pH 3.31. A measure of the response of the fiber film to zinc ions is the difference in the 620 nm PL at 30 min (when zinc ions are added) and at 35 min in Figure 10. Such a response is plotted as a function of the pH value in Figure 10E. It suddenly drops to almost zero as the pH changes from 3.31 to 2.38; the sensor therefore may not work well in very acid parts of the body such as the stomach.

4. Conclusion

In conclusion, a solid-state sensing film is developed to detect zinc II ions within the physiological concentration range in complex liquid medium. The fluorescent molecular probe embedded in the hydrogel polymer host responds exclusively to zinc ions, even when many other ions and proteins are present in the medium. The film is made in fiber form by an electrospinning method in order to raise the sensitivity. In the medium

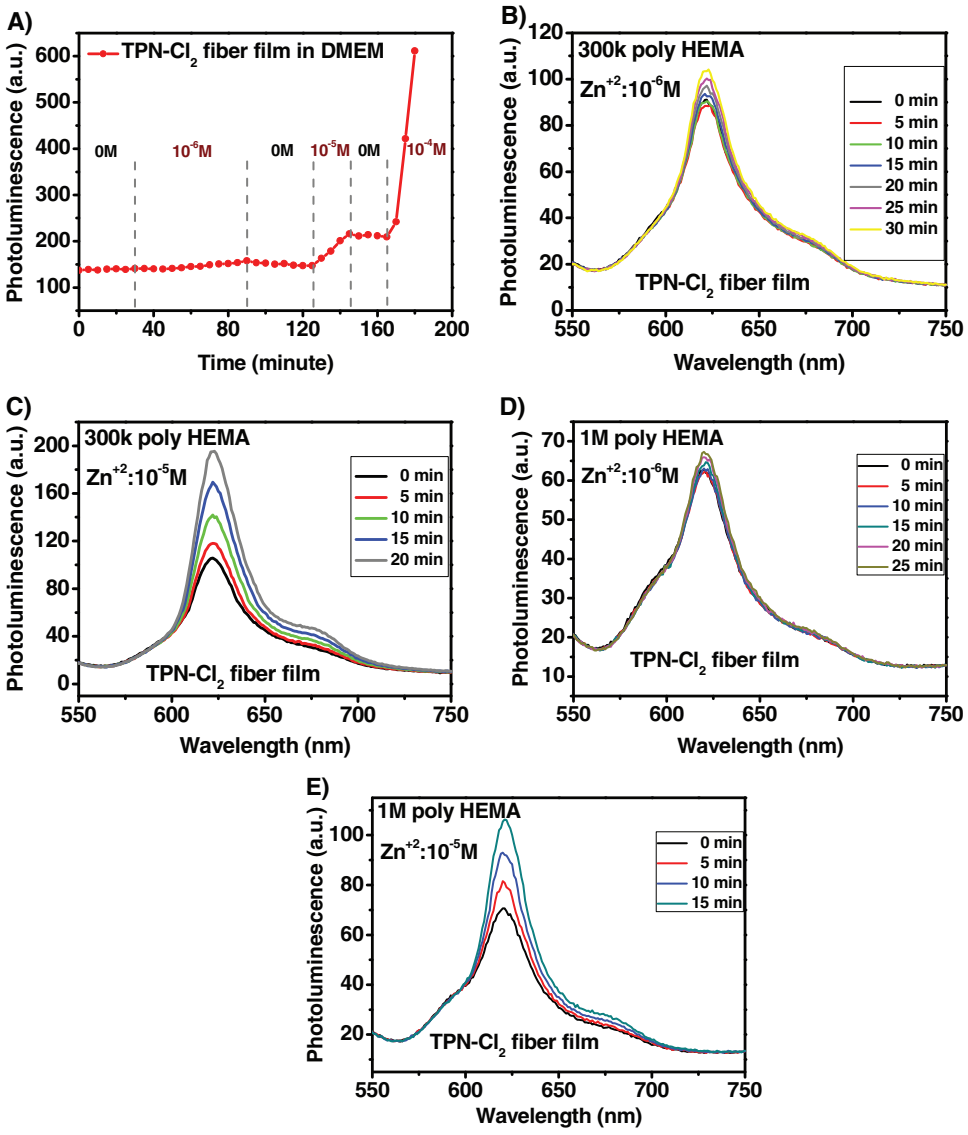

Figure 9. A) The time-dependent PL spectrum taken at 620 nm. TPN-Cl_2 fiber film works well in pure DMEM solution. The time-dependent PL was recorded at 620 nm, measured by a PL spectrometer. B–E) The response of the PL spectra to zinc ions, for fiber films of 300 k and 1 M poly HEMA molecular weight in DMEM solution.

such a film respond to zinc ions with concentrations as low as 10^{-6} M with a time resolution of 5 min. The fiber film shows good stability and unchanged sensing functions in high acidic conditions. Zinc ion plays crucial roles in brain function and immune system. The sensitivity and respond speed of the film makes it possible for a non-invasive and real-time study for the variation of zinc ion concentration in important biological processes.

Acknowledgements

J.H.S. and H.F.M. thank Yu-Fan Chang for useful discussions. This work was supported by the Ministry of Economic Affairs of Taiwan under Contract No. 99-EC-17-A-07-S1-157, National Science Council of Taiwan under Contract No. 99-2628-M-009-001- and No.98-2112-M-007-028-MY3, Veterans General Hospitals and University System of Taiwan Joint Research Program(VGHUST100-G5-1-1), and the Academic Sinica under Contract No. AS-100-TP-B03. The authors are grateful to the members in the Biomimetic System Research Center at National Chiao Tung University.

Received: May 7, 2012
Revised: July 6, 2012
Published online: October 23, 2012

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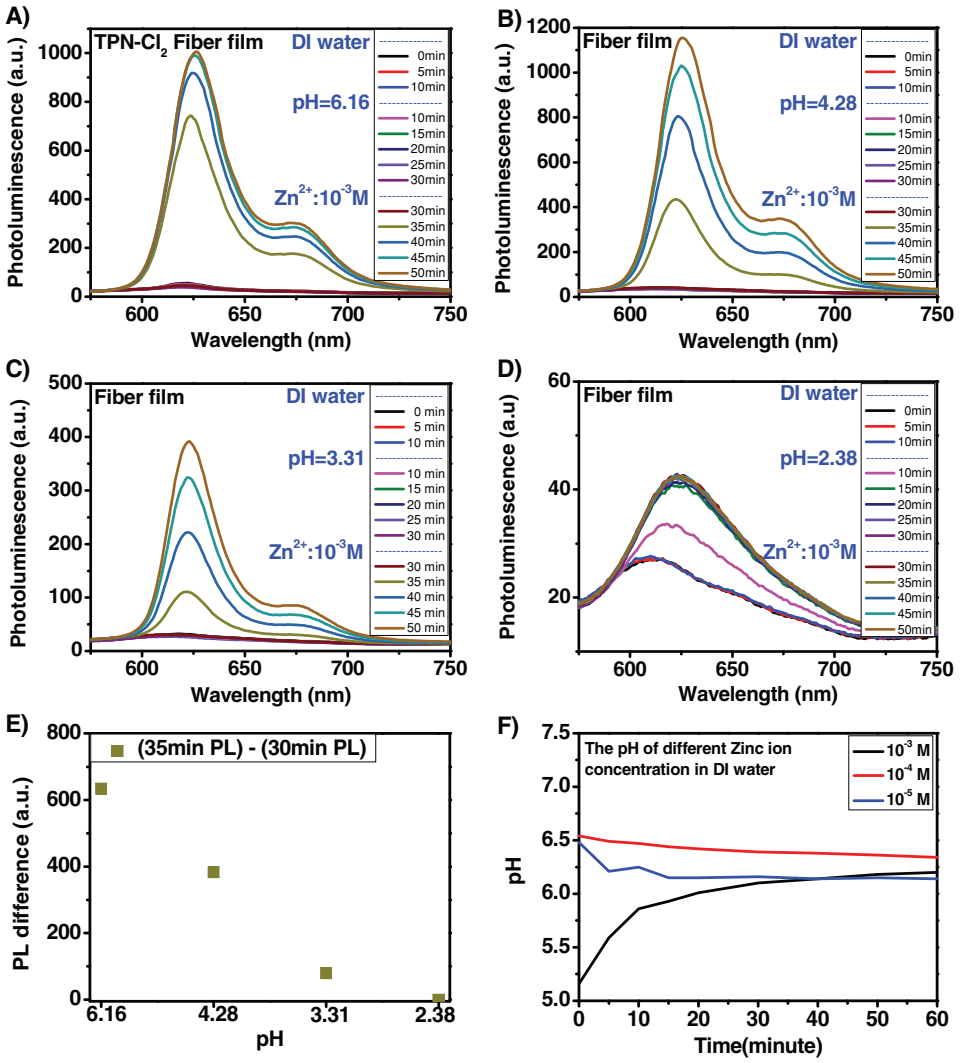

Figure 10. The PL spectra at different times for various pH values in DI water: A) pH = 6.16; B) pH = 4.28; C) pH = 3.31; and D) pH = 2.38. E) The difference in PL signal at 620 nm at 35 min and 30 min as a measure of response to zinc ions for various pH values. F) The variation of the water pH value after addition of zinc acetate at three concentrations.

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